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EFFECTS OF TAO-HUNG-TANG ON HAEMORHEOLOGICAL DISTURBANCE INDUCED BY THROMBUS FORMATION IN RATS

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TAO-HUNG-TANG(THT) is widely used for activating blood circulation and removing blood stasis in Chinese medicine. The purpose of this study was to investigate the effects of THT on haemorheological variables (hematocrit, plasma viscosity, whole blood viscosity, red cell aggregation and red cell deformability) and the plasma fibrinogen concentration were determined in blood sample from rats with ligature in the inferior vena cava (IVC) for 15 hours. The effects of THT on thrombus formation induced by ligating the IVC for 2 hours in rats was also studied. The thrombus weight, incidence of thrombosis and blood clotting factors (prothrombin time and partial thromboplastin time) and plasma fibrinogen concentration were examined. THT (0.75; 3.0 g/kg) inhibited the thrombus formation significantly. For blood-clotting factors, THT had no effect on prothrombin time and partial prothromboplastin time, but reduced the fibrinogen concentration at a dose of 0.75 g/kg THT. As for haemorheological parameters, THT decreased the whole blood viscosity at every shear rate but had no obvious effect on plasma viscosity. It also reduced the mean corpuscular hemoglobin concentration and enhanced red cell deformability. It is postulated that the inhibition of thrombus formation in rats by THT may be related to erythrocyte disaggregation. Furthermore, THT might act on erythrocyte aggregation and deformability to improve haemorheological disturbance in rats.

Key Words: Tao-Hung-Tang, Haemorheological Disturbance, Thrombus Formation, Erythrocyte Deformability.

INTRODUCTION

Thrombosis and its complications, such as cerebral ischemia and myocardial infarction, are the leading causes of death in many countries. Recently, it has been suggested that thrombotic diseases be associated with blood hyperviscosity syndromes[1] which refer to a group of symptoms and signs related to increased blood viscosity[2]. Blood hyperviscosity not only aggravates the development of disease but also threatens a patient's life. To date, there is no breakthrough and remains controversial in clinical treatment[3]. However, the traditional Chinese medicine has already developed a unique medication[4] to prevent and cure the disorders of circulatory system. Many Chinese physicians use the crude drugs of activating blood circulation and removing blood stasis to heal cerebral embolism, coronary heart disease, headache and abnormal menstruation, etc. Their prescriptions always contain four basic crude drugs, Persical semen, Carthami flos, Chuanxiong rhizoma and Veitchii radix. The effects of each or combination of these four crude drugs on antiplatelet aggregation and fibrinolysis in vitro has been studied[5,6], but not on haemorheology. However, haemorheology also seems to play a very important role in the process of thrombus formation, and this can not be neglected. Therefore, we combined these four crude drugs and named it Tao-Hung-Tang(THT) according to the Golden Mirror of Medicine[7] and investigated its effect on

haemorheology in vivo. We followed Reyer's method[8] to disturb blood rheology in rats with experimentally induced thrombus formation. All parameters of haemorheology, RBC aggregation and deformability, plasma and whole blood viscosity, hematocrit, and the plasma fibrinogen concentration were measured[9]. From the present study, we hope to provide a new direction in researching the pharmacological action or mechanism of these four kinds of crude drugs.

MATERIALS AND METHODS

Preparation of Tao-Hung-Tang

The mixture(1 kg) of powders of Persical semene(Rosaceae), Carthami flos(Compositae), Ligustici chuanxiong rhizoma(Umbelliferae), Paeoniae veitchii radix (Ranunculaceae), at equal proportion, was immersed in 5000 ml of 95% alcohol at room temperature for 3 days and then extracted in a water bath at a temperature below 80°C for several times until the solution was clear. The extract was evaporated and concentrated in a rotary vacuum evaporator at a temperature just below 60°C. The sample was obtained by Lyophilizing the concentrated extract. When given to rats, the preparation was dissolved in water.

Animals

Male Sprague-Dawley rats weighing between 250 and 350 g were used in all experiments.

Thrombus formation and haemorheological alteration

We followed Reyer's method[8] to induce thrombus formation. and also to disturb the haemorheological properties by ligating the inferior vena cava in rats for 15 hours.

Drug and treatment regimens

(1) Thrombus formation

Three different doses of THT(0.75 g/kg; 1.5 g/kg; 3.0 g/kg) and a single dose of aspirin (10 mg/kg) were given orally to rats once a day for 7 consecutive day[10]. On the 7th day, ligation of the inferior vena cava was performed one hour after drug administration.

(2) Haemorheological alteration

THT was given orally to rats like what we mentioned above. But, on the 6th day, the operation was performed before administration. Then, after ligature for 2 and 14 hours, preparations were given to rats once again, respectively. All rats were sacrificed and blood was withdrawn from the abdominal artery for measuring haemorheological parameters 15 hours after ligature.

Blood sample

Blood was withdrawn from the abdominal aorta of rats into EDTA tube. Plasma was obtained by centrifuging whole blood at 3000 rpm for 10 min.

All rheological measurements and haematological examinations were performed within 2 hours of blood collection.

Rheological Parameters.

(1) Viscosity: The viscosity of whole blood and plasma was measured at various shear rates

at 37°C with a cone-plate viscometer(Biorheolizer, Tokyo Keiki Co., Ltd., Tokyo).

(2) Erythrocyte deformation: RBC deformability was estimated from viscosity measurements using the blood viscosity equation of Dintenfass[11] and expressed as TK value.

$TK = (\tilde{n}^{0.4} - 1)/\tilde{n}_r^{0.4} \cdot Ht$

T: Talor factor ñ: apparent whole blood viscosity

K: erythrocyte aggregate index Ht: Hematocrit

$\tilde{n}_r$: relative viscosity(whole blood viscosity/plasma viscosity)

Hematological parameters

Hematological indices, including hematocrit, mean corpuscular hemoglobin concentration and number of erythrocytes, were measured by ultralogic 800 hematology analyzer.

Measurement of blood-clotting factors

The measurement method of plasma prothrombin time and partial thromboplastin time was in accordance with Proctor and Rapaport[12] and Quick[13]. Fibrinogen concentration was measured according to the method of Tomikawa et al.[14] and Lowry et al.[15]

Statistical analysis

Data were expressed as mean ± S.E. and analyzed by the analysis of unpaired Student's t-test with a significant level of $P < 0.05$.

RESULTS

As shown in Table 1, the mean weight of the thrombi was increased with prolonging ligature time. The incidence of thrombosis 2 hours after ligature was 83; whereas the incidence of thrombosis at other periods(4, 6 and 8 hrs) of ligature was 100 percent. Blood viscosity was significantly increased at every shear rate at 8, 15 and 24 hours of ligature, but not for 2 hours ligature at high shear rate(Fig. 1). The TK value, representing the RBC deformability, was elevated appreciably in the groups with ligature for 15 and 24 hours.

The thrombus formation was inhibited markedly by THT at doses of 0.75 and 3.0 g/kg, while aspirin-treated group appeared no obvious changes (Table 2).

As Shown in Table 3, THT did not affect prothrombin time and partial thromboplastin time, but the former was prolonged in aspirin-treated group. THT obviously decreased the plasma fibrinogen concentration at a dose of 0.75 g/kg. THT had a tendency to normalize hematocrit value and the mean corpuscular hemoglobin concentration(MCHC) which was elevated in control group (Table 4). TK value was decreased at doses of 0.75 and 3.00 g/kg. At ever shear rate, blood viscosity, especially the low-shear viscosity, decreased at dose of 0.75 g/kg(Fig. 2).

Table 1 Incidence of thrombus formation and thrombus weight 2 hours after ligature of the inferior vena cava in rats.

Ligature time (h.)	Thrombus weight (mg)	Incidence of thrombosis (%)
2	4.7±0.7	83%
4	6.6±0.4	100%
6	7.9±0.8	100%
8	9.5±1.5	100%

Values are expressed as mean ± S.E.M. in six rats.

Table 2 Incidence of thrombus formation and thrombus weight 2 hours after ligature of the inferior vena cava in experiment rat and in rats given the different doses of THT.

Groups	Thrombus weight (mg)	Incidence of thrombosis (%)
experiment	3.0±0.8	83%
THT 0.75 g/kg	0***	0%
1.50 g/kg	1.6±0.5	50%
3.00 g/kg	1.0±0.6*	67%
Aspirin 10 mg/kg	2.8±0.7	83%

Values are expressed as the mean ± S.E.M.(n=6).
Statistically significant differences: *P<0.05, ***P<0.001(THT vs experiment).

Table 3 Effect of THT on blood-clotting factors after experimentally induced thrombus formation in rats(2 hrs).

Groups	PT(sec.)	PTT(sec.)	Fibrinogen(mg)
Normal	9.1±0.4	17.4±0.4	234±5
Experiment	9.5±0.3	16.9±0.7	276±5
THT 0.75 g/kg	9.0±0.4	16.5±0.4	253±6*
1.50 g/kg	9.0±0.4	16.5±0.9	265±5
3.00 g/kg	9.1±0.5	16.5±0.5	266±4
Aspirin 10 mg/kg	10.1±0.2*	18.2±0.3	263±4

Values are expressed as mean ± S.E.M. in six rats.
Statistically significant differences: *P<0.05(vs experiment).
Experiment: Ligature but no administration of THT.

Table 4 Effect of THT on hematological factors after experimentally induced thrombus formation in rats (15 hrs).

Groups	RBC ($10^6/\mu l$)	Hematocrit (%)	MCHC (g/dl)	TK	Fibrinogen (mg)	Plasma viscosity (mPa/s)
Normal	6.4±0.4	39.6 ±0.7	33.1 ±0.7	0.9 ±0.1	234±5	1.3±0.1
experiment	5.6±0.6	31.1 ±1.5***	39.0 ±1.7**	1.1± 0.1	394 ±5***	1.2±0.1
THT 0.75g/kg	6.0±0.4	37.4 ±1.6*	33.5 ±0.7*	0.8± 0.1*	415±6*	1.4±0.1
THT 1.50g/kg	6.1±0.7	36.2 ±1.7*	33.0 ±1.0*	0.9± 0.1	488±6*	1.3±0.2
THT 3.00g/kg	5.1±0.5	34.3 ±1.3	33.6 ±0.8*	0.9± 0.1*	439±3*	1.3±0.1

Values are expressed as mean ± S.E.M. in six rats.
Statistically significant differences: *P<0.05(THT vs experiment), **P<0.01, ***P<0.001(experiment vs normal).
Experiment: Ligature but no administration of THT.

DISCUSSION

Blood hyperviscosity has been regarded as a risk factor in cerebral and cardiovascular diseases. There are four major determinants of blood viscosity, red cell deformability, fibrinogen, hematocrit and plasma viscosity. Drugs affecting those parameters may improve the blood fluidity. In particular, enhancing red cell deformability may activate infusion in the microcirculation. Investigating the pharmacological action of THT from the viewpoint of haemorheology is to open new approaches for the therapy of circulatory diseases.

In the present study, we established, firstly, an animal model of haemorheological disturbance by ligating the inferior vena cava in rats in accordance with Reyer's method. Secondly, we used it to investigate the effects of THT on preventing thrombus formation and improving haemorheological disturbance. The thrombus weight 2 hours after ligature was nearly half of that in 15 hours. The blood viscosity and TK value 15 hours after ligature were higher than those in 24 hours. Therefore, ligature for 2 and 15 hours were chosen to be the time course for evaluating the effects of THT on antithrombosis and haemorheology.

Thrombi usually originate from the regions with the lowest blood flow, such as the venous valve pocket, postcapillary venules or deep veins. In the low shear(low flow) vein-vascular area, an increased tendency of red cell aggregation is easily present. Basically, the formation of red cell aggregates, either normal or pathological, is a shear rate-dependent phenomenon which is considerably augmented if the forces of flow resulting from the haemodynamic pressure are reduced below their normal value[18]. The primary cause leading to such a reduction as systemic hypotension, heart failure, and vascular stenoses, etc., is the initiation of a negative feedback of flow retardation, enhanced red cells aggregation, increased blood viscosity, and

further reduced blood flow until permanent stasis emerges, resulting in a rheological occlusion[1,18]. Thereafter, red cells are no longer deformed, and become less deformable because of stagnant hypoxia and acidosis[1], and this will contribute to the danger of microcirculatory impairment. The experimental evidence of increases in blood viscosity at every shear rate and in TK value, expressed as red cell deformability, shown in Fig 1, is in agreement with what we mentioned above. The longer the pathological state exists in vivo, the worse the vicious cycle of haemorheological condition goes. Aspirin prolongs clotting time by inhibiting platelet aggregation[19]. It becomes the major cause of itself to induce bleeding sideeffect. In our present study, THT(0.75 g/kg) showed more effectively than aspirin (10 mg/kg) on antithrombus formation. It also did not affect the clotting factors of PT and PTT. According to the results, it implies that THT might be different from aspirin in their action mechanism. But it needs further investigation. At low shear rates, the progressively increased viscosity is primarily related to fibrinogen-induced red cell aggregation[20]. The reduction in low shear blood viscosity is considered to result from decreased red cell aggregation[21]. Therefore, the significantly antithrombus formation of THT(0.75 g/kg) might be related to its reducing both factors of fibrinogen concentration and low-shear blood viscosity. In the microcirculation, red cell deformability is very important for maintaining the blood fluidity. The erythrocyte has to change its shape continuously in response to the driving pressures and to the vessel geometry in order to fulfil its function in the capillary bed[22]. At high shear-rates, red cell deformability is the predominant property determining the viscosity, especially in pathological state[20]. The erythrocyte deformability is reduced during cell aging, a phenomenon that may reflect alterations in intracellular viscosity(mainly due to mean corpuscular hemoglobin concentration; MCHC)[23], membrane rigidity or red cell shape. They are also the three major determinants of red cell deformability[24]. THT(0.75: 1.5 g/kg) decreasing blood viscosity at every shear rate may be concerned with reducing hematocrit; increasing red cell deformability may be related to normalize MCHC, respectively. In other words, the effects of THT on blood viscosity, hematocrit and MCHC contribute to improving haemorheological disturbance. Although plasma viscosity is mainly dependent on plasma protein, especially fibrinogen[1], the increased fibrinogen concentration had no positive effect on plasma viscosity in this study. In addition, THT had no dose-dependent effect in all experiments.

Blood is a complicate multiphase system. The blood viscosity measured in vitro or in vivo is only a gross indication. Therefore, haemorheological factors should be considered internationally and analyzed concretely. Increase in low-shear blood viscosity in vitro reflects that red cell aggregates in vivo; increase in high-shear blood viscosity in vitro reflects that red cell deformability decreases in vivo. The mechanism of THT decreasing the low and high shear blood viscosity, either to stabilize the charge on red cell membrane or to alter the intracellular viscosity, still needs further investigation.

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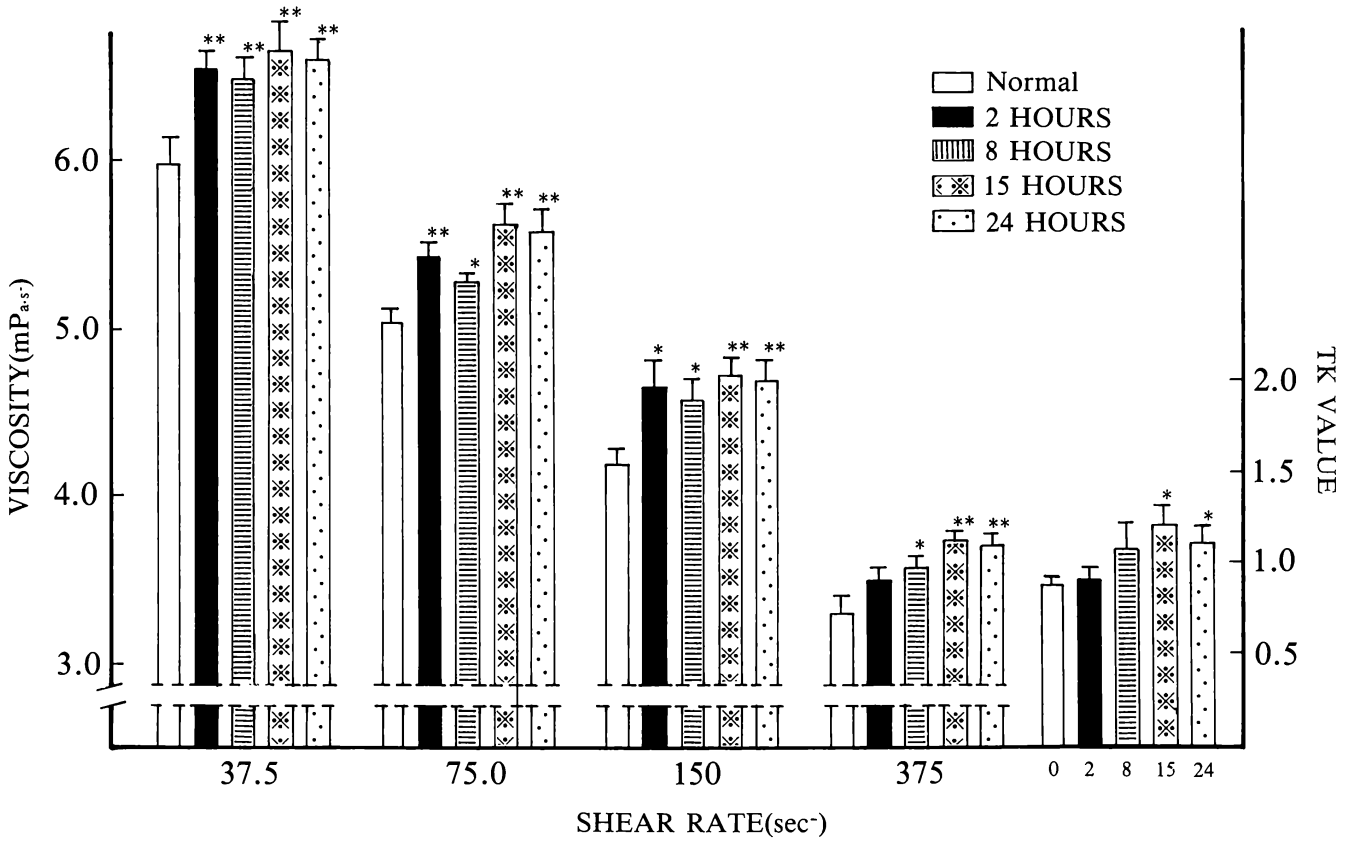

Fig. 1 Alteration of blood viscosity after experimentally induced thrombus formation in rats. Each column represents the mean value of six experiments. Statistically significant differences: *P<0.05, **P<0.01(vs normal).

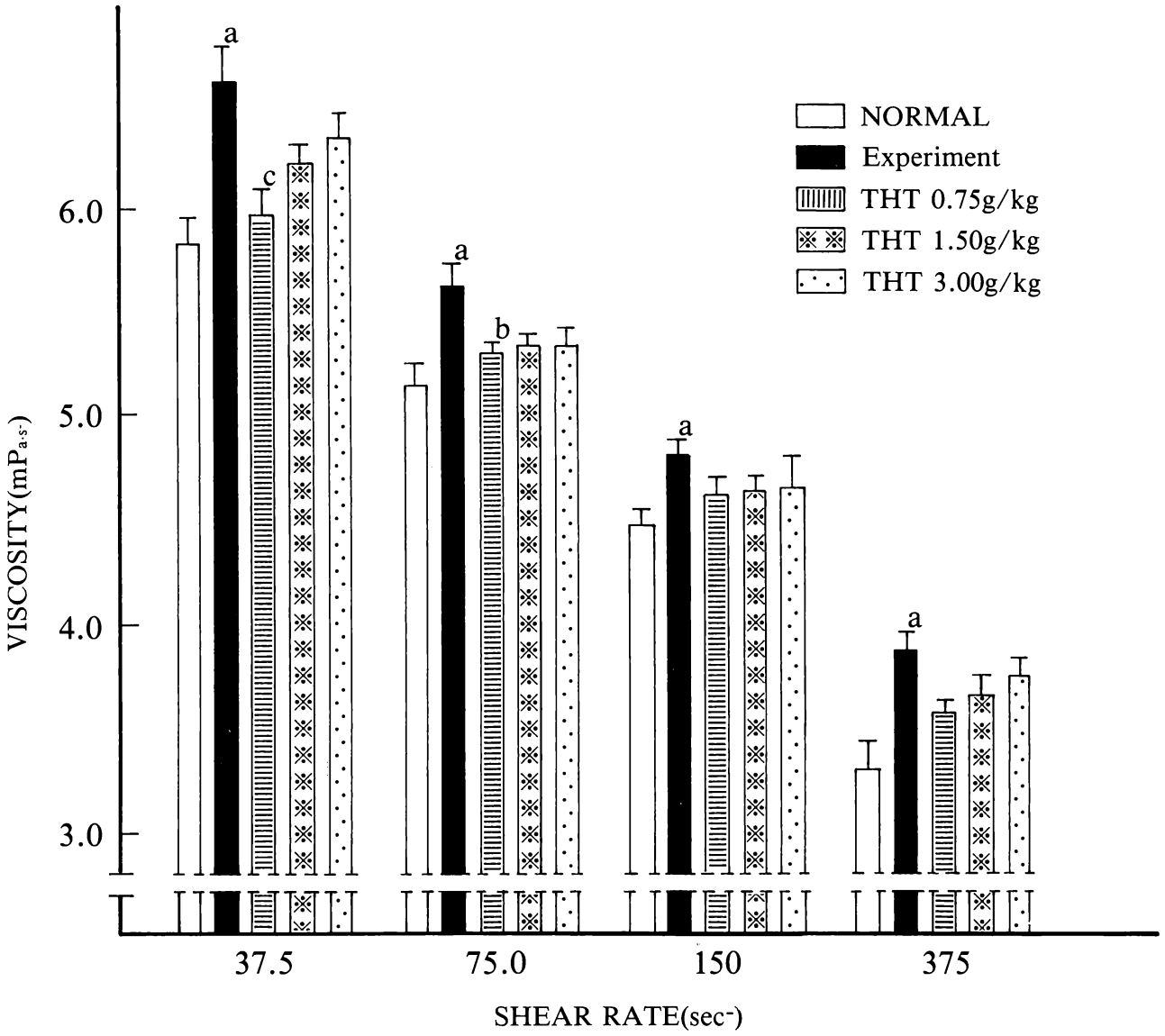

Fig. 2 Effects of THT on blood viscosity in rat experimentally induced thrombus formation. Each column represents the mean value of six experiments. Bars denote the standard error.
Statistically significant differences: a)$P<0.01$(experiment vs normal), b) $P<0.05$, c) $P<0.01$(THT vs experiment).

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桃紅湯對於大鼠血栓所誘發血液流變性障礙之影響

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血栓症（ thrombosis ）及其所誘發的腦中風和心肌梗塞等心腦血管疾病，始終佔居國內十大死亡原因的前位。據研究指出血栓性疾病常有“血液高黏症綜合症（ blood hyperviscosity syndrome ）”的發生，不僅會加深病情的惡化，更會對患者的生命產生威脅。然而，臨床上至今對於血栓性疾病和血液高黏滯症的治療，尚無突破性發展。但在我國中醫學上，早已發展獨特的活血化瘀療法，有效的防治血栓性疾病的發生和發展。在活血化瘀方劑中，常應用桃仁、紅花、川芎和赤芍等四味基本活血化瘀藥材的配伍。有關這四種關單味藥或任一配伍在抗血小板聚集和體外纖溶作用的研究，早有報告。唯有關這四味藥配伍對於血液流變學的影響，尚未見研究報告。因此，我們將此四味藥合成方劑而名之爲桃紅湯，並探討其對血液流變性障礙之影響，冀從中醫藥的領域中，能尋得具有預防血栓和改善血液黏度的藥物。

本實驗以大鼠下腔靜脈結紮的方式誘導血栓形成及血液流變性異常狀態，並依血液流變學的理論，測定影響血液黏滯性的因子：紅血球聚集性和變形性（ red cell aggregation and deformability ）、血球比容積（ hematocrit ）、血漿和全血黏度（ plasma and whole blood viscosity ）和血漿纖維蛋白原濃度（ plasma fibrinogen concentration ）等各項血液流變指標，以探討桃紅湯對於血液流變性的影響及其機轉。結果獲至如下：(1)桃紅湯以 0.75g/kg 和 3.00g/kg 對血栓形成的抑制作用最明顯。(2)桃紅湯（ 0.75g/kg ）能減少血漿纖維蛋白原濃度。(3)桃紅湯（ 0.75g/kg ）能降低各切速的全血黏度值，但對血漿黏度沒有明顯的影響。(4)桃紅湯能降低平均血球血紅素濃度和表紅血球變形能力的 TK 值。

由本實驗的結果顯示，桃紅湯能有效的抑制大鼠體內血栓的形成並且改善因血栓所誘發的血液流變性異常的情形。這些作用可能是桃紅湯影響紅血球聚集性和變形性二項流變因子所致。對於其能增強紅血球變形功能的作用機理，是否因爲穩定紅血球細胞膜表面電荷或是改善紅血球內黏度（ 主要取決於平均血球血紅素濃度 ），則有待更進一步的研究證實。

關鍵詞：桃紅湯，血液流變性障礙，血栓形成，紅血球變形性。